Control of Drug-to-antibody Ratio in Cysteine-based Conjugation ADCs

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The Drug-to-antibody ratio (DAR) is crucial for Antibody-Drug Conjugates (ADCs), affecting drug efficacy, potency, and safety. This poster outlines DAR control strategies for traditional cysteine conjugation offering insights into optimizing therapeutic outcomes.

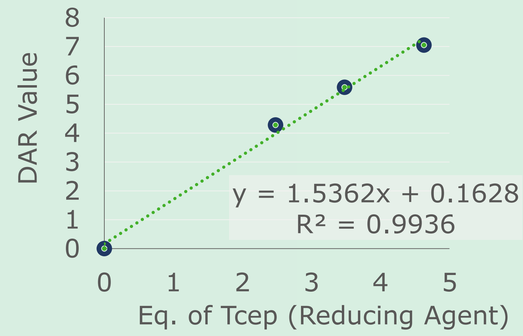
Case Study: Adcetris®

- Conjugation Type: Interchain reduced cysteine
- Antibody: Brentuximab (Produced by Mycenx)
- Linker-Payload: Vedotin (vc-MMAE)
- Target Drug-Antibody-Ratio (DAR) value: 4

01// Hit Target DAR value

A test was conducted using three different equivalents (eq.) of the reducing agent, TCEP, to establish a linear regression model for investigating the relationship between DAR (Drug-to-Antibody Ratio) and the equivalent amount of the reducing agent.

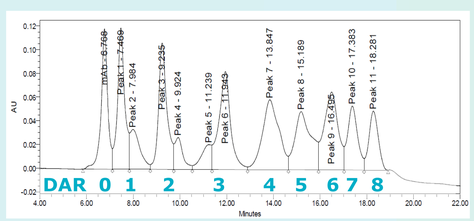
In this study, it was determined that to achieve a DAR of 4, 2.5 equivalents of the reducing agent are required.



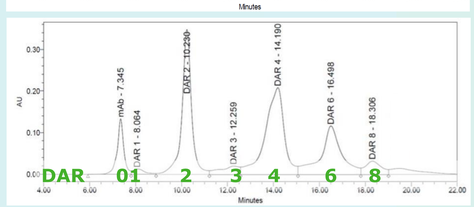
02// Minimize the Heterogeneity of DAR Distribution

The DAR represents the average outcome of an ADC. Minimizing the heterogeneity in DAR distribution, especially for those odd DAR species, is important for safety considerations, as free thiol groups may potentially interfere with critical biophysical activities. In this study, adjusting the equivalent ratio of the payload and could effectively reduce the heterogeneity in DAR distribution.

Eq. ratio of payload/Tcep: **1.5**
- DAR Average:4
- High heterogeneity of DAR Distribution



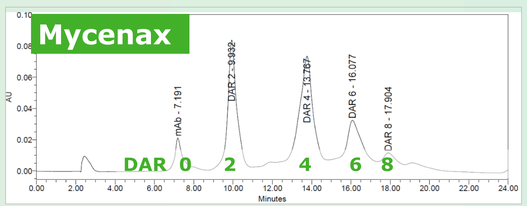
Eq. ratio of payload/Tcep: **2**
- DAR Average:4
- Low heterogeneity of DAR Distribution

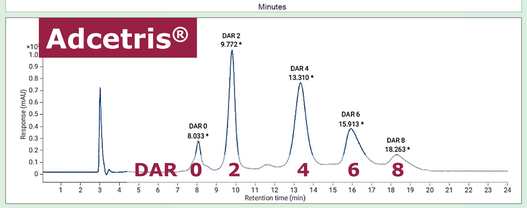


03// Generate a Adcetris® Biosimilar

We implemented additional process refinements, focusing on optimizing temperature and reaction time, to minimize the presence of odd DAR species. Furthermore, the DAR of the ADC sample, prepared using the process developed by Mycenax, closely resembles that of Adcetris®.

Sample	DAR Distribution (%)					DAR avg.
	0	2	4	6	8	
Mycenax	5.8	27.0	**35.2**	20.9	11.0	4.1
Adcetris®	6.9	25.1	**35.8**	23.3	8.9	4.0

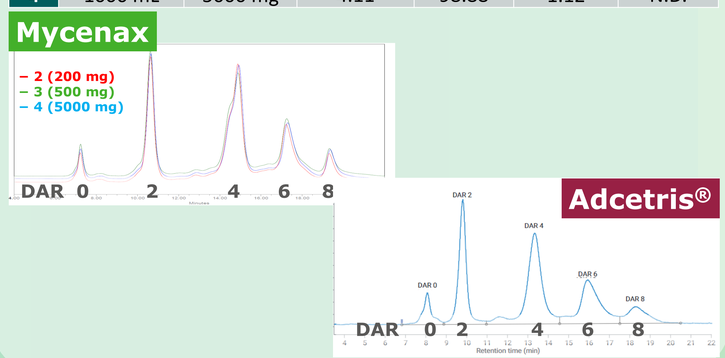




04// Scale-up Study from 7.5mg to 5g

After developing a suitable process for producing an Adcetris® biosimilar, we conducted a stepwise scale-up study, ranging from 7.5 mg to 5 g. Remarkably, we observed a consistent pattern in the distribution of the Drug-to-Antibody Ratio (DAR) across different scales.

No.	Volume (mL)	Amount (mg)	DAR (HIC-LC)	Purity	HMWS	LMWS
				SE-HPLC (%)		
1	1.5 mL	7.5 mg	4.12	98.87	1.13	N.D.
2	40 mL	200 mg	4.09	98.76	1.24	N.D.
3	100 mL	500 mg	4.11	98.48	1.35	0.18
4	1000 mL	5000 mg	4.11	98.88	1.12	N.D.



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